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Herpes Simplex Virus-Induced Stromal Keratitis: Role of T-Lymphocyte Subsets in Immunopathology

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Herpetic stromal keratitis (SK), a frequent cause of visual impairment, is considered to represent an immune-mediated inflammatory response to persistent herpes simplex virus virions or subcomponents within the corneal stroma. The experimental disease in mice involves the essential participation of T lymphocytes, but the role of T-lymphocyte subsets in either mediating or controlling the disease is uncertain. In this report, rat monoclonal antibodies were used to selectively deplete mice in vivo of $CD4^+$ (helper-inducer) and $CD8^+$ (cytotoxic-suppressor) T-cell populations and the effect on herpetic SK was evaluated. As measured by flow cytometry, mice treated with anti-CD4 monoclonal antibody (GK 1.5) were >95% depleted of $CD4^+$ T lymphocytes and mice treated with anti-CD8 monoclonal antibody (2.43) were 90% depleted of $CD8^+$ T lymphocytes. Depleted and nonspecific mouse ascites-treated control mice were infected topically on the corneas with herpes simplex virus type 1, and the induction of various immune parameters during the acute infection was evaluated. $CD4^+$-depleted mice failed to produce either a significant antiviral antibody or delayed-type hypersensitivity response but were capable of producing normal cytotoxic T-lymphocyte responses. In contrast, $CD8^+$-depleted mice produced antiviral antibody and delayed-type hypersensitivity responses comparable with those in control animals, but cytotoxic T-lymphocyte responses were markedly reduced. Clinical observations of the corneas revealed that SK in $CD4^+$-depleted mice was significantly reduced, whereas in $CD8^+$-depleted mice SK developed more rapidly, was more severe, and involved a greater percentage of mice. These observations implicate the $CD4^+$ T-lymphocyte subset as the principal mediators of SK and $CD8^+$ T lymphocytes as possible regulators that control the severity of SK.

Infection of the eye with herpes simplex virus (HSV) can cause a variety of syndromes, but perhaps the most significant, because it frequently causes permanent vision impairment, is stromal keratitis (SK), which is associated with recurrent episodes of HSV infection (for a review, see reference 26). Although the exact nature of the pathogenesis of SK remains unresolved, much evidence supports the involvement of an immune-mediated inflammatory process (3, 6, 12, 17–19, 30, 33; J. F. Metcalf, Am. Vis. Res. Org. Proc., abstr. no. 200, 1983). Thus, whereas antiviral agents are usually ineffective in the treatment of SK (8), this disease often responds well to immunosuppressive therapy (2). The notion that SK represents an immune-mediated chronic inflammatory response to persisting nonreplicating viral components or corneal neoantigens receives much support, but there is less certainty about the mechanistic nature of the immunopathological reaction (for a review, see reference 26). Studies on experimental animal models of SK support a role for immune complexes and polymorphonuclear neutrophils (19), as well as immunopathology mediated by T cells (9, 12, 17, 27; Metcalf, Am. Vis. Res. Org. Proc., 1983). Initial evidence supporting the latter idea came from Metcalf's laboratory, which showed that athymic mice, unlike their euthymic littermates, failed to develop SK upon ocular infection with HSV (17). Subsequently, Russell and coworkers (27) confirmed the observations of Metcalf et al. and further implicated a role for T cells in SK by showing that the lesions occurred if athymic mice were reconstituted with T cells, in particular with T cells from HSV-immune animals. Whether or not one or both major phenotypic subsets of T cells ($CD8^+$ and $CD4^+$) are involved in the immunopathological process remains ill defined. Accordingly, Russell et al. (27) initially suggested the involvement of class I major histocompatibility complex-restricted cytotoxic T lymphocytes (CTL) in SK. This notion was later supported by Diefenbach and co-workers (C. G. Diefenbach, R. L. Hendricks, and J. C. Glorioso, Abstr. Annu. Meet. Assoc. Res. Vis. Ophthalmol. 1988, abstr. no. 1, p. 151) on the basis of their studies with HSV mutants that were purportedly poor inducers of the CTL response and subsequently poor inducers of SK. Others have implicated the delayed-type hypersensitivity (DTH) reaction, which in the case of HSV is mediated by $CD4^+$ T cells (16, 22, 23), in SK immunopathology (12, 26, 30, 33). In the present report, we have readdressed the question of the role of one or the other of the two major phenotypic subsets of T lymphocytes in the immunopathology of SK by taking advantage of the recently available immunosurgical approach, in which mice can be selectively depleted of a given T-cell subset by the in vivo administration of monoclonal antibody to their appropriate surface antigen (4, 22). Our results implicate the $CD4^+$ cells as principally involved in the SK immune pathology. Moreover, we also demonstrate an essential role for $CD8^+$ lymphocytes in controlling the severity of SK.

MATERIALS AND METHODS

Virus. HSV type 1 (HSV-1) strain RE was grown on Vero cells supplemented with McCoy 5A medium with 10% heat-inactivated donor calf serum (GIBCO Laboratories, Grand Island, N.Y.), penicillin (100 U/ml), and streptomycin (100 μg/ml). A recombinant vaccinia virus containing the cloned gene for the influenza virus (A/JAP/305/57) hemagglutinin molecule was grown in HEp-2 cells and purified as described previously (5). Virus was titered on Vero cells, and the 50%

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tissue culture infective dose ($TCID_{50}$) per milliliter was determined at 5 days after infection. Virus was aliquoted in small amounts and stored at −70°C. A new aliquot of titered virus was thawed before use in subsequent experiments.

Mice. Male BALB/c AnNHsdBR and female C3H HenHsd (Harlan Sprague Dawley, Indianapolis, Ind.) mice 6 to 8 weeks of age were used throughout the experiments. Surgical procedures were carried out by using the general inhalant anesthetic methoxyflurane (Pitman-Moore, Washington Crossing, N.J.).

Corneal inoculation. Inoculations on anesthetized mice were achieved by proptosing the eye and slightly abrading the cornea, in a crisscross pattern, with a 28-gauge needle (Becton Dickinson and Co., Rutherford, N.J.). A 4-μl portion of virus containing 10^6 $TCID_{50}$s was then placed on the corneal surface and gently massaged into the eye with the eyelids.

Monoclonal antibodies and T-cell subset depletion. The GK 1.5 hybridoma was obtained from the American Type Culture Collection, Rockville, Md. This cell line secretes rat monoclonal antibody reactive with the murine T-cell surface antigen CD4 (L3T4), expressed on the helper-inducer T-cell subsets (31). The 2.43 hybridoma was a generous gift of Frank Fitch (University of Chicago, Chicago, Ill.) and has been described previously (28). This cell line secretes rat monoclonal antibody reactive with the murine T-cell surface antigen CD8 (Lyt 2.2), expressed on BALB/c T cells.

Mice treated with anti-CD4, anti-CD8, or nonspecific mouse ascites (NMA) were inoculated intravenously on days −3, −2, and +2, with day 0 representing the day of HSV-1 corneal challenge. Mice were then reinoculated intraperitoneally with the same volume of antibody on days +7, +14, +21, and +28 to maintain the depleted state. The total amount of specific antibody in this volume of ascites, as determined by a modified enzyme-linked immunosorbent assay method, was 3 mg for anti-CD4 and 0.1 mg for anti-CD8.

Flow cytometry. Cells from spleen and lymph nodes of individual mice were obtained at various times throughout the experiments and incubated on ice for 1 h with a 1/5,000 dilution of heat-inactivated (56°C for 30 min) 50% ammonium sulfate-precipitated anti-CD4 or anti-CD8 antibodies in RPMI 1640 medium containing 2% heat-inactivated fetal bovine serum (GIBCO) and 0.2% sodium azide (Fisher Scientific Company, Fair Lawn, N.Y.). This was followed by three washes in the RPMI 1640 medium and second-step staining with fluorescein-conjugated goat anti-rat antibody (Southern Biotechnology Associates, Birmingham, Ala.). Cells were again washed three times, and single-color fluorescence analysis was performed by using a FACStar PLUS fluorescence-activated cell sorter (Becton Dickinson).

Clinical and pathological observations. Severity of SK was determined by observations with a slit lamp biomicroscope (Keeler Instruments Broomall, Pa.) and based on the following scoring system: 0, no disease; 1+, slight corneal opacity; 2+, moderate opacity; 3+, severe opacity with the lens visible; 4+, opaque cornea; 5+, severe necrotizing keratitis. Scores were determined with the reader unaware of which mice belonged to which treatment group.

Tissues for light-microscope examination were fixed in 10% phosphate-buffered Formalin for 72 h and then embedded in paraffin wax. Twelve coronal sections per eye were then stained with hematoxylin and eosin.

DTH assay. Five mice from each group were assayed for their ability to mount a DTH response. Mice were challenged by injecting a 50-μl solution, containing 1.5×10^6 $TCID_{50}$s of UV-inactivated HSV-1 or vaccinia virus, into the right rear footpad. The left rear footpads received a 50-μl solution containing UV-inactivated HEp-2 cell lysates. Mice whose corneas were abraded and which were inoculated with phosphate-buffered saline served as an additional negative control. Footpad swelling was measured 24 h after challenge with spring-loaded calipers (Dyer Co., Lancaster, Pa.). The amount of nonspecific swelling in the control left footpad was subtracted from that in the right rear footpad for final quantitation of the DTH response.

Antibody determination. Serum collected from animals at 38 days postinfection was heat inactivated (56°C for 20 min) and diluted in twofold dilutions, and for each dilution, 50 μl was aliquoted into 96-well flat-bottom microtiter plates (Costar, Cambridge, Mass.). To each well, 20 μl of medium containing 150 $TCID_{50}$s of the RE strain of HSV-1 was added, and the sealed plates were incubated at 4°C for 24 h. Each serum sample was represented in a minimum of six replicates. To one-half of the replicates, 50 μl of Low Tox rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada) diluted 1/4 was added, while the other replicates received 50 μl of medium. Plates were then resealed and incubated at 37°C for 1 h. Next, 10^4 Vero cells in a 50-μl solution were added to each well, and the plates were resealed and incubated for 5 days at 37°C. At 4 and 5 days later, wells were examined for cytopathic effect and the serum titers were determined as the dilution that neutralized 50% of the virus, by the method of Spearman and Karber (7).

CTL assay. CTL cultures were generated by the method of Pfizenmaier et al. (25). Briefly, 5 days after the inoculation of 10^6 $TCID_{50}$s of HSV-1 KOS into the rear footpads, the draining popliteal lymph nodes were removed and single-cell suspensions were prepared. Viable cells were adjusted to 2×10^6/ml in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (heat inactivated at 56°C for 20 min)–7 mM L-glutamine–5×10^{-5} M 2-mercaptoethanol and cultured at 37°C as 20-μl aliquots in 100-mm^2 petri dishes (Becton Dickinson Labware, Rutherford, N.J.). After 3 days of culture, cells were washed and viable cells were used as CTL effectors in a standard 4-h chromium release assay as described previously (13).

RESULTS

Phenotype of spleen and lymph node cells from mice treated with relevant antisera. To determine the effectiveness of antiserum treatment on depletion of T-lymphocyte subsets, spleen and lymph node cells from test and control animals were analyzed for $CD4^+$ and $CD8^+$ cells by flow cytometry. The data in Fig. 1 are representative and depict profiles of spleen cells at 7 days following corneal challenge with HSV-1 and after 3 administrations of anti-T lymphocyte ascites or NMA. Typically, $CD4^+$ cells were >95% depleted and $CD8^+$ cells were >90% depleted. In the experiments, mice were given repeat injections of the relevant antisera on days 7, 14, 21, and 28 to maintain the depleted state. Profiles of test animals in various groups taken at the termination of experiments, on day 38, were similar to those shown in Fig. 1 (data not shown).

Effect of T-cell depletion on the induction of DTH, production of neutralizing anti-HSV-1 antibody, CTL response, and central nervous system (CNS) disease. At 35 days postinfection, UV-inactivated HSV-1 was injected into one rear footpad of each of five mice from each of the treatment groups to test for DTH reactivity. As seen in Fig. 2, depletion of $CD4^+$-bearing T cells significantly reduced the

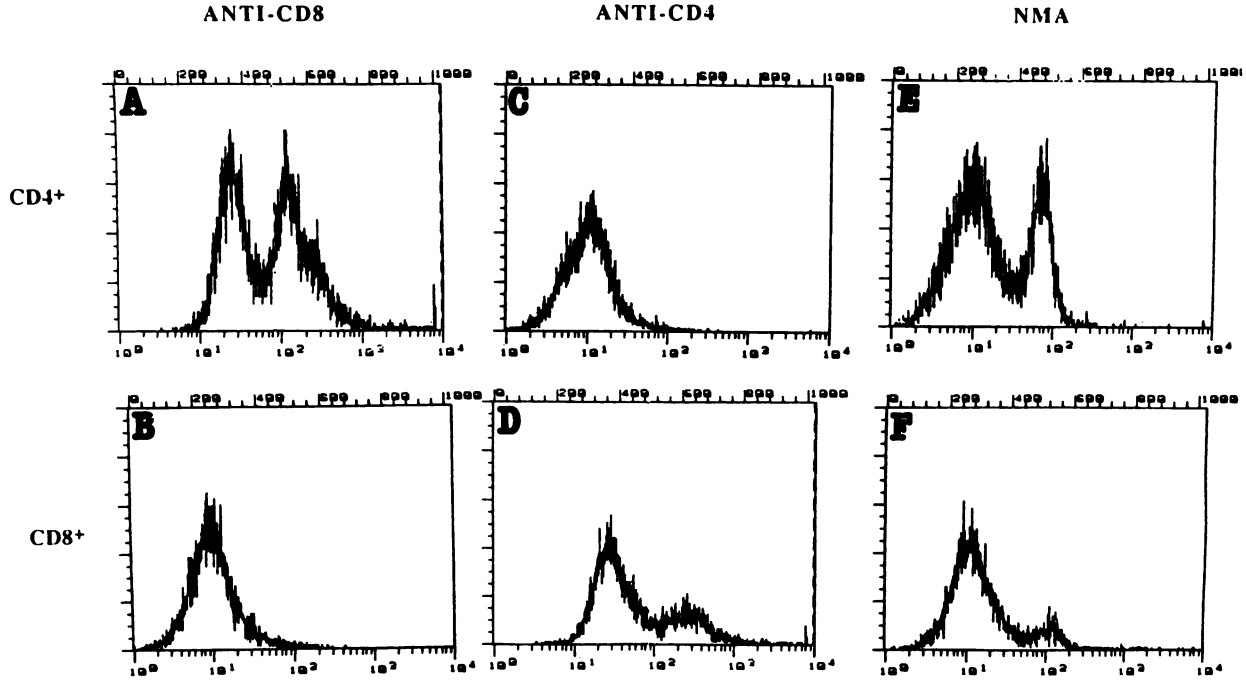

FIG. 1. In vivo depletion of T-cell subsets after anti-CD4 or anti-CD8 treatment. Cells from spleens of individual mice which received antiserum treatment on days −3, −2, and +2 (with day 0 representing the day of HSV-1 corneal challenge) were obtained on day 7. Cells were then stained and analyzed for the presence of $CD4^+$ and $CD8^+$ T lymphocytes by flow cytometry. (A) Normal $CD4^+$ population of T lymphocytes from mice treated with anti-CD8 monoclonal antibody. (B) Depletion of the $CD8^+$ T-lymphocyte subset following anti-CD8 treatment. (C) Depletion of the $CD4^+$ T-lymphocyte subset following anti-CD4 treatment. (D) Normal $CD8^+$ population of T lymphocytes from mice treated with anti-CD4. (E) Normal $CD4^+$ population of T lymphocytes from mice treated with NMA. (F) Normal $CD8^+$ population of T lymphocytes from mice treated with NMA.

extent of virus-specific DTH, whereas depletion of $CD8^+$ T cells had no effect upon the extent of DTH.

At 38 days postinfection, the mice described above were bled from the retro-orbital sinuses and the HSV-1-specific antibody response was determined. The data shown in Table 1 demonstrate that whereas $CD8^+$ T-cell depletion had no effect upon the levels of antiviral antibodies, mice depleted of $CD4^+$ T cells produced either marginal or undetectable anti-HSV-1 neutralizing antibody. In accordance with previous reports (22), the in vivo depletion of $CD4^+$-bearing T lymphocytes prior to the inoculation of HSV-1 did not abrogate the subsequent development of HSV-1-specific CTL (Fig. 3). Conversely, the HSV-1-specific CTL response was completely inhibited in $CD8^+$-depleted mice (Fig. 4).

An interesting observation was that although mice depleted of $CD4^+$ T lymphocytes subsequently lost the ability to mount a significant DTH or neutralizing antibody response, all animals remained healthy throughout the HSV-1 challenge experiments. In contrast, in some experiments, mice depleted of $CD8^+$ T lymphocytes lost weight, had hind-limb paralysis, and subsequently died within 2 weeks of initiation of the experiment (Fig. 5).

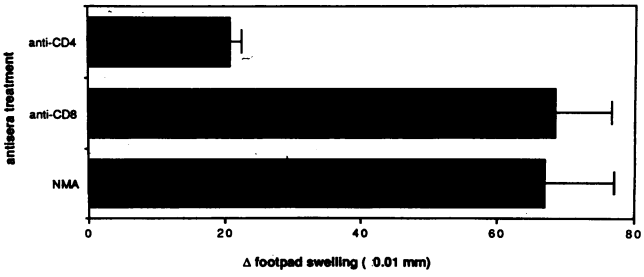

FIG. 2. Effect of in vivo T-lymphocyte depletion on the generation of an HSV-specific DTH response. At 35 days postinfection, five mice from each treatment group were challenged in the right rear footpad with 1.5×10^6 $TCID_{50}$s of UV-inactivated virus. The left rear footpads received an equal volume of HEp-2 cell lysate. Footpad swellings were measured with spring-loaded calipers at 24 h after challenge. The amount of background swelling of the control left footpad was subtracted from the amount of swelling observed in the test right footpad. Data are expressed as the mean of results from five mice plus or minus one standard error. For anti-CD4 versus NMA, $P < 0.005$.

Comparison of corneal stromal disease in intact and $CD4^+$- and $CD8^+$-depleted mice. Mice were examined for SK by slit lamp biomicroscopy approximately every 2 days after infec-

TABLE 1. Levels of neutralizing antibodies in intact and T-cell-subset-depleted mice

Antiserum treatment	Inverse of neutralization titers[a]	
	− Complement	+ Complement
Anti-CD4	<5	54 ± 7[b]
Anti-CD8	25 ± 0.5	2,284 ± 497
NMA	37 ± 9	2,980 ± 639

[a] Titers were measured at 38 days after corneal challenge with HSV-1 and are expressed as means plus or minus the standard error of five mice per group. Five preimmune mice served as a negative control, and their neutralization titers were below the detection limits of the assay. Serum collected from mice immunized subcutaneously with HSV-1 had neutralization titers of 35 without (−) complement and greater than 7,000 with (+) complement.

[b] Significantly less than NMA control ($P < 0.005$).

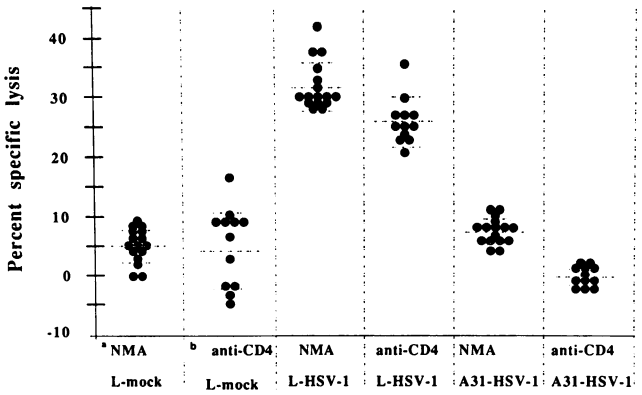

FIG. 3. HSV-1-specific CTL activity in intact and $CD4^+$-depleted mice. Lymphocytes which were either mock (NMA) or $CD4^+$ (GK 1.5) depleted were obtained from the popliteal lymph nodes of acutely infected C3H HenHsd mice and were cultured as described previously and used as a source of CTL effectors in a 4-h chromium release assay. Target cells were infected with the respective viruses shown (multiplicity of infection, 5) and labeled with $Na_2{}^{51}CrO_4$ (200 μCi) for 4 h prior to the assay. Target cells were autologous L929 cells ($H\text{-}2^k$) or allogeneic A31 cells ($H\text{-}2^d$). CTL populations were used at an effector-to-target ratio of 30:1, and percent specific lysis was determined as described previously (13). Results are shown as individual values of 16 NMA (a) and 12 CD4-depleted (b) replicate cultures, with the broken lines representing the mean plus or minus one standard deviation.

tion. Epithelial damage was observed by 2 days postinfection in all mice inoculated with HSV-1. This was normally followed by a mild inflammatory reaction which resolved soon thereafter. Peripheral corneal neovascularization and stromal infiltration were evident within approximately 1 week in those mice which developed SK. All mice in the NMA control group and the $CD8^+$-depleted group developed various stages of SK within 3 weeks postinfection, and the majority of these animals developed the more intense forms of SK. In contrast, throughout the entire experiment a

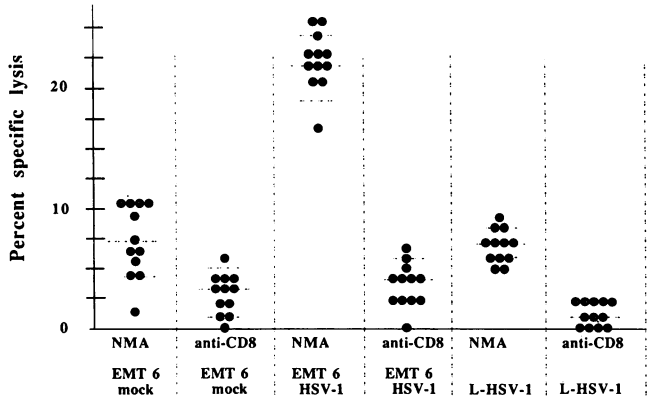

FIG. 4. Loss of HSV-1-specific CTL activity in $CD8^+$-depleted mice. Lymphocytes which were either mock (NMA) or $CD8^+$ (2.43) depleted were obtained from the popliteal lymph nodes of acutely infected BALB/c mice and were cultured as described previously as a source of CTL effectors in a 4-h chromium release assay, as described in the legend to Fig. 3. Target cells were autologous EMT6 cells ($H\text{-}2^d$) or allogeneic L929 cells ($H\text{-}2^k$). CTL populations were used at an effector-to-target ratio of 50:1. Results are shown as individual values of 12 replicate cultures, with the broken lines representing the mean plus or minus one standard deviation.

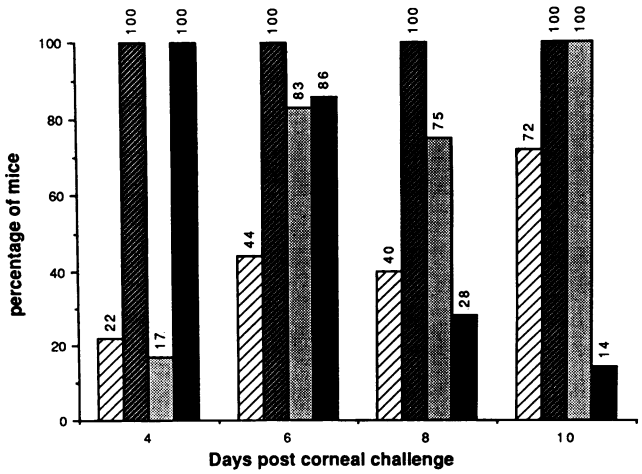

FIG. 5. Susceptibility of $CD8^+$ T-cell-depleted mice to CNS disease following HSV-1 corneal challenge. BALB/c mice were inoculated with 0.100 mg of anti-CD8 (2.43) monoclonal antibodies or nonspecific mouse ascites on days −3, −2, and +2. Corneas were abraded in a checkerboard configuration with a 28-gauge needle prior to topical corneal inoculation with 10^6 $TCID_{50}$s of the RE strain of HSV-1 on day 0. Mice were then inoculated on day 7 with the monoclonal antibody to prolong the depleted state. In some experiments, during examination for stromal disease, animals displayed signs of CNS disease such as weight loss, hind-limb paralysis, and eventually death. ▨, Percentage of mice with SK following treatment with NMA; ▨, percentage of mice which survived HSV-1 corneal challenge following treatment with NMA; □, percentage of mice with SK following treatment with anti-CD8; ■, percentage of mice which survived HSV-1 corneal challenge following treatment with anti-CD8.

maximum of 30% of the mice in the $CD4^+$-depleted group developed SK. The initiation of SK in $CD8^+$-depleted mice was comparable to that in the NMA group, but the disease in the $CD8^+$-depleted group was more severe and developed more rapidly (Table 2).

Random mice were chosen from each group at the termination of the experiments, and eyes were taken for histological examination. The corneas from mice treated with NMA, as well as from control mice, were heavily infiltrated with both mononuclear and polymorphonuclear leukocytes. However, corneas from $CD4^+$-depleted mice showed only mild inflammatory responses. In the $CD8^+$-depleted mice, corneal inflammatory responses were similar to those of the control group. On the basis of the severity of SK in this treatment group (as determined by slit lamp microscopy), we expected a more intense infiltration in these mice, but this was not evident as judged histologically.

In Fig. 6 and 7, a compilation of the average severity of SK for all mice in each of the treatment groups is presented. The results indicate that removal of $CD4^+$ T cells markedly reduced the immunopathology associated with herpetic SK, whereas removal of T cells expressing the $CD8^+$ phenotype resulted in an enhanced severity of SK.

DISCUSSION

In most circumstances, disease associated with HSV infection results from cytolytic effects of the virus and therapy with antiviral drugs represents a valuable approach. However, in a few situations, immunopathological responses to persisting virus or viral antigens account for the

TABLE 2. Development of HSV-induced SK after in vivo depletion of T-cell subsets

Days postinfection and severity[a]	No. of mice at SK stage/total no. of mice (% at SK stage) with treatment		
	NMA	Anti-CD8	Anti-CD4
7			
0	8/10 (80)	9/16 (56)	9/10 (90)
+1	2/10 (20)	6/16 (38)	1/10 (10)
+2	0/0 (0)	0/0 (0)	0/0 (0)
+3	0/0 (0)	1/16 (6)	0/0 (0)
+4	0/0 (0)	0/0 (0)	0/0 (0)
+5	0/0 (0)	0/0 (0)	0/0 (0)
14			
0	1/10 (10)	2/16 (12.5)	7/10 (70)
+1	2/10 (20)	0/0 (0)	0/0 (0)
+2	3/10 (30)	2/16 (12.5)	0/0 (0)
+3	0/0 (0)	0/0 (0)	3/10 (30)
+4	0/0 (0)	0/0 (0)	0/0 (0)
+5	4/10 (40)	12/16 (75)	0/0 (0)
21			
0	1/10 (10)	1/16 (6.3)	7/10 (70)
+1	2/10 (20)	2/16 (12.5)	0/0 (0)
+2	1/10 (10)	0/0 (0)	0/0 (0)
+3	0/0 (0)	0/0 (0)	0/0 (0)
+4	1/10 (10)	0/0 (0)	3/10 (30)
+5	5/10 (50)	13/16 (81.3)	0/0 (0)
28			
0	2/10 (20)	1/16 (6)	7/10 (70)
+1	1/20 (10)	0/0 (0)	0/0 (0)
+2	1/10 (10)	0/0 (0)	0/0 (0)
+3	0/0 (0)	0/0 (0)	0/0 (0)
+4	0/0 (0)	0/0 (0)	2/10 (20)
+5	6/10 (60)	15/16 (94)	1/10 (10)

[a] Severity of SK was determined by observations with a slit lamp biomicroscope and based on the following scoring system: 0, no disease; 1+, slight corneal opacity; 2+, moderate opacity; 3+, severe opacity with the lens visible; 4+, opaque cornea; 5+, severe necrotizing keratitis.

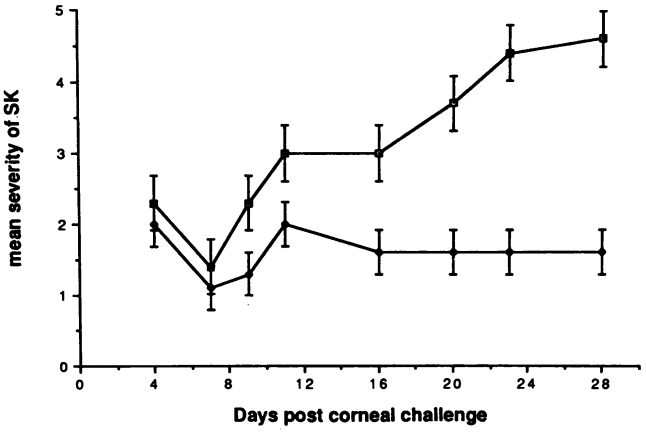

FIG. 6. Therapy with anti-CD4 reduced the severity of stromal keratitis. BALB/c mice were inoculated with 3 mg of anti-CD4 (GK 1.5) monoclonal antibody (◆) or nonspecific mouse ascites (⊡) on days −3, −2, and +2. Corneas were abraded in a checkerboard configuration with a 28-gauge needle prior to topical corneal inoculation with 10^6 $TCID_{50}$s of the RE strain of HSV-1 on day 0. Mice were then inoculated once per week with the monoclonal antibody to prolong the depleted state. Eyes were examined for stromal disease with a slit lamp biomicroscope, and scores were determined as previously described. Day 20 anti-CD4 versus NMA, $P < 0.01$; day 23 anti-CD4 versus NMA, $P < 0.0005$.

lesions and strategies for therapeutic intervention must differ. By far the most significant syndrome which is assumed to result from an immunopathological response to HSV is SK, a disease that is among the most common causes of visual impairment in the United States (26). Our present report provides evidence for the immunopathological nature of SK and further demonstrates that T cells of the $CD4^+$ phenotype play an essential role in the process. Accordingly, whereas infection of nondepleted animals with HSV-1 via the cornea was followed by stromal lesions, lesions were absent or markedly diminished in mice depleted of $CD4^+$ lymphocytes by the in vivo administration of anti-CD4 serum. In fact, although the majority of nondepleted mice developed severe SK, only a minor proportion of the mice in the $CD4^+$-depleted group developed SK, and in these mice the lesions were less severe in nature. Presumably, these lesions evident in some $CD4^+$-depleted mice were accounted for by the approximately 5% of $CD4^+$ cells that remain after the in vivo antiserum therapy. However, it is possible that more than one mechanism of immunopathology could account for the SK lesions, as has been discussed earlier (26). Some have suggested that SK could represent an immune-complex lesion with polymorphonuclear leukocytes principally involved in the inflammatory response (19). Our results cannot exclude a role for toxic immune complexes, but we favor the hypothesis that the mechanism by which the stromal reaction is carried out is by means of $CD4^+$ cells that mediate a DTH reaction (16, 23). Thus $CD4^+$-suppressed mice failed to produce a DTH response to HSV, and previous observations had demonstrated that the inability of athymic mice to generate SK could be reconstituted by adoptive transfers of T cells capable of mediating DTH (M. P. Nasisse, R. G. Russell, D. W. Horohov, and B. T. Rouse, Abstr. Annu. Meet. Assoc. Res. Vis. Ophthalmol. 1984, abstr. no. 35, p. 25). The results of experiments on split

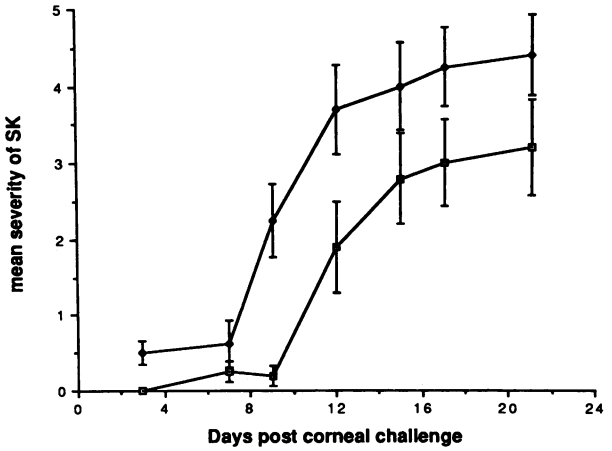

FIG. 7. Therapy with anti-CD8 increased the severity of SK. BALB/c mice were inoculated with 0.100 μg of anti-CD8 (2.43) monoclonal antibodies (◆) or nonspecific mouse ascites (⊡) on days −3, −2, and +2. Corneas were abraded in a checkerboard configuration with a 28-gauge needle prior to topical corneal inoculation with 10^6 $TCID_{50}$s of the RE strain of HSV-1 on day 0. Mice were then inoculated once per week with the monoclonal antibody to prolong the depleted state. Eyes were examined for stromal disease with a slit lamp biomicroscope, and scores were determined as previously described. Day 9 anti-CD8 versus NMA, $P < 0.005$; day 12 anti-CD8 versus NMA, $P < 0.05$.

tolerance also substantiate an essential role for DTH in SK (discussed subsequently), and the essentially mononuclear cell nature of the stromal reactions resembles that of DTH rather than immune-complex lesions.

Immunopathology in SK resulting from a cytotoxic T-lymphocyte-mediated event has also been suggested (27). Support came from the results of adoptive transfer experiments, as well from the observation that gC mutant HSV viruses that failed to act as CTL targets were also incapable of causing stromal reactions following corneal infection of AJ (*H-2*^{a}) mice. Our present report, however, provides no support for a role of CD8$^+$ major histocompatibility complex class I restricted CTL in the immunopathological reaction of BALB/c (*H-2*^{d}) mice. Accordingly, vigorous SK reactions still occurred in mice depleted of CD8$^+$ lymphocytes. These mice were also determined to be markedly diminished in their HSV CTL responsiveness. Indeed, although the onset of SK in CD8$^+$-depleted mice was similar to that in control mice, its severity was, on the average, increased in these animals. After a period of 28 days, virtually all mice in the latter group developed severe necrotizing keratitis and, in addition, many developed symptoms of CNS disease. Consequently, it appears that rather than mediating immunopathology, CD8$^+$ cells perform a protective role. How such a role is mediated needs definition, but on the basis of previous reports by others (14, 15) as well as ourselves (11), we favor the idea that the protective effect by CD8$^+$ cells is mediated at least in part by their known suppressor cell activity, which, in this instance, is acting against the CD4$^+$-mediated DTH response. A similar protective effect was demonstrated by Altmann and Blyth (1) by using a model in which an immunopathological reaction against HSV occurred in the CNS which appeared, on the basis of adoptive transfers, to be mediated by a DTH reaction that caused demyelination of sensory nerves. In that study, they demonstrated that suppression of the HSV-DTH response by T suppressor cells inhibited the immunopathological damage. Many others have also demonstrated that the HSV-specific DTH reaction is subject to modulation by suppressor cells at either the afferent or efferent level (21, 29). In our CD8$^+$-depleted mice, we could still demonstrate potent DTH reactions although, perhaps unexpectedly, these were not increased in magnitude over those observed in nonsuppressed mice. However, subtle increases in activity might be observed in CD8$^+$-depleted mice if measured by more analytical assays. We are currently attempting to perform such analysis by using an adoptive transfer approach.

The importance of confirming the nature of the pathogenesis of SK and of establishing the mechanism of its immunopathology lies with the ultimate strategy chosen for treatment and prevention of this important disease. Consequently, since CD4$^+$ and CD8$^+$ cells show different restriction specificities and appear to respond to different fragmentation products of target proteins (20), any vaccine designed to stimulate or suppress these subsets must take such facts into consideration. In addition, strategies are known in which the immune response induced may differ in terms of the array of immunological activities generated. Thus, in the HSV system, several approaches result in a negative DTH reaction but a positive response in all other measured aspects of immunity. This so-called split tolerance can be produced either by immunizing mice with virus by the intravenous (24), intraperitoneal (10), or intracameral (32) route or by exposure of mice to certain anti-idiotype reagents (11). In this latter circumstance, we have demonstrated that exposure of mice to a heterologous anti-idiotype serum produced against a monoclonal anti-glycoprotein C antibody causes a suppressor-cell-mediated inhibition of the DTH reaction to HSV. Moreover, the use of such an anti-idiotype reagent in mice renders them suppressed in their ability to generate an immunopathological SK following corneal infection with HSV (S. Martin, unpublished data). The use of the anti-idiotype reagent, as well as other means of selectively modulating CD4 cell function, is currently under investigation as a means of modulating the SK reaction against HSV.

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